STUDIES ON THE ESTERASE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM OF THE FRESH-WATER MOLLUSC (ANODONTA CYGNEA)

by

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Enzymological studies on the central nervous system of the fresh-water mollusc have been carried out so far only from morphological and biochemical aspects (Baranyi 1954, Baranyi – Saláneki 1963, Domján – Minker 1963). Histochemical studies have been conducted first of all with acid and alcaline phosphateses under normal conditions (Baranyi 1966, Lane 1963, Nagy 1968,). Ábrahám and his co-workers (Ábrahám – Minker 1959, Domján – Minker 1963, Minker – Domján 1961) have used biochemical methods for the determination of acid and alkaline phosphatase activities in the central nervous system of the fresh-water molluscs. Dahl and his co-workers (Dahl – Falk – Mecklenburg – Myhrberg – Rosengren 1966) examined the effect of dopamine of the central nervous system of *Anodonta piscinalis*. In addition to the use of conventional morphological and biochemical techniques some authors made an attempt to scale down their assay method, maintaining, at the same time, its quantitative character (Friede – Knoller 1965, Glick 1957, Roodyn 1965). The aim of our experiments was to determine the changes in esterase activity under the effect of low and high temperatures. In order to increase the accuracy of our measurements the "extenction method" has been modified.

Materials and Methods

Thirty six fresh water molluscs with 3–4 annual rings were used. The animals had been kept at 15 to 16 °C for 8 days before the start of the experiments.

The animals were divided in the following way:

1. Control animals: 12 animals were divided into four groups and kept at 15–16 °C throughout the experiments.

2. 12 animals were divided into 4 groups and kept at 5 °C for 24, 48, 72, and 96 hours, respectively.
3. 12 animals were divided into four groups and kept at 24 °C for 24, 48, 72 and 96 hours, respectively.

All animals were kept under the same illumination (electric bulb, 25 W, 220 V, from a distance of 150 cm). The experiments were conducted in June.

From the animals belonging to these groups cerebral, visceral and pedal ganglia were taken under indentical experimental conditions. In our experiments the activity of the specific cholinesterase, as well as that of the nonspecific esterases present in the ganglia, such as alpha esterase, AS esterase and lipase were determined. In the assay of choline esterase the ganglia kept at 20 °C for two hours in 10% formalin, and then for 20 hours in 4% formalin, in a refrigerator.

For the determination of alpha esterase and AS esterase activities the ganglia were fixed in 10% formalin for two hourst at 20°C. In the assay of lipase the ganglia were fixed in 4 percent formalin for 24 hours. After fixing, frozen sections, 100μ thick, were prepared. Esterases were assayed by the following methods:

Choline esterase according to the Koelle – Friedenwald-method (K o e l l e – F r i e d e n w a l d 1949) As a substrate acetylthiocholine iodide was used.

Alpha esterase according to the method of Seligman, with naphthyl acetate as substrate.

AS esterase according to Martin's method (M a r t i n 1953, with naphthol – AS as substrate.

Lipase according to Gömöri's method (P e a r s e 1960), with Tween-60 as substrate.

The sections were examined morphologically on the one hand, and on the other hand their light-transmission was measured with the Jurányi extinctionmeter. By suing the latter method enzyme activities could be expressed quantitatively.

In the extinction measurements the diaphragm was adjusted to 5 cm. The sections were placed in a casette-system which was built in facing the light source. First the light transmission of the slides was measured, and then that of the sections. The light transmission of the slides was used as a correction factor. The size and shape of the sections prepared from the ganglia were different. To correct these differences, the measured values were expressed as enzyme activity/mm^2. The measurements were made in a range of 4000 – 8000 Å with readings at 500 Å intervals. With each animal esterase activity was measured in both right and left ganglia.

The aritmetical means of the six data obtained in this way were plotted and evaluated by planimetry. The data calculated were plotted again.

Results

The sections were 100 μ tchick, and the cell-structure was not seen. Therefore only the intensity of the colour-reaction indicating esterase activity the treated tissues could be compared to tha of the control. The brown colour characteristic of AS esterase activity was lighter in the sections prepared from the animals kept at 5 °C, than in those of the control animals. After a treatment for 24 hours the colour characteristic of lipase activity faded then it grew more intense, again. In the sections of the anglia from animals kept at 24 °C only the colour indicating lipase activity decreased in intensity. In the sections prepared from the visceral ganglia, neither the changes induced by low, not those induced by high temperature could be registered by mikroscopic examinations. The pedal ganglia generally showed reduced enzyme activity upon cold or warm treatment. As shown by the result of light-microscopy there was no visible difference between the effects of treatment lasting 24, 48, 72, or 96 hours.

Results obtained by extinction measurements. The activities of the specific and non specific esterases in all three ganglia changed as a function of time. These changes involved in some cases an increase and in some others a decrease in enzyme activity.

A) Cold treatment brought about the following changes in the cerebral ganglia (Fig. 1)

Choline esterase. As a result of cold treatment the activity of choline esterase varied significantly according to rhythm of 24 hours.

Alpha esterase. During the first 3 days the activity of alpha esterase increased, and on the fourth day it reached the normal level.

A*S esterase.* The values were below the normal level, and on the last day the activity dropped to n. zero.

Lipase. The activity decreased on the first day significantly, then it increased suddenly as a function of time.

B) Changes in the cerebral ganglia as induced by heat treatment (Fig. 2)

Choline esterase. The enzyme activity was first around the normal value, then it creased.

Alpha esterase. The activity first decreased significantly, then it increased; in 72 hours it reached the maximum, and in 96 hours it was reduced to the normal level.

AS esterase. The enzyme activity was below the normal value.

Lipase. The activity was significantly below that of the control

C) Changes in the visceral ganglia as induced by cold treatment Fig. 3)

Choline esterase. The activity changed significantly as a funktion of time it dropped to zero after 48 hours, and then it increased again.

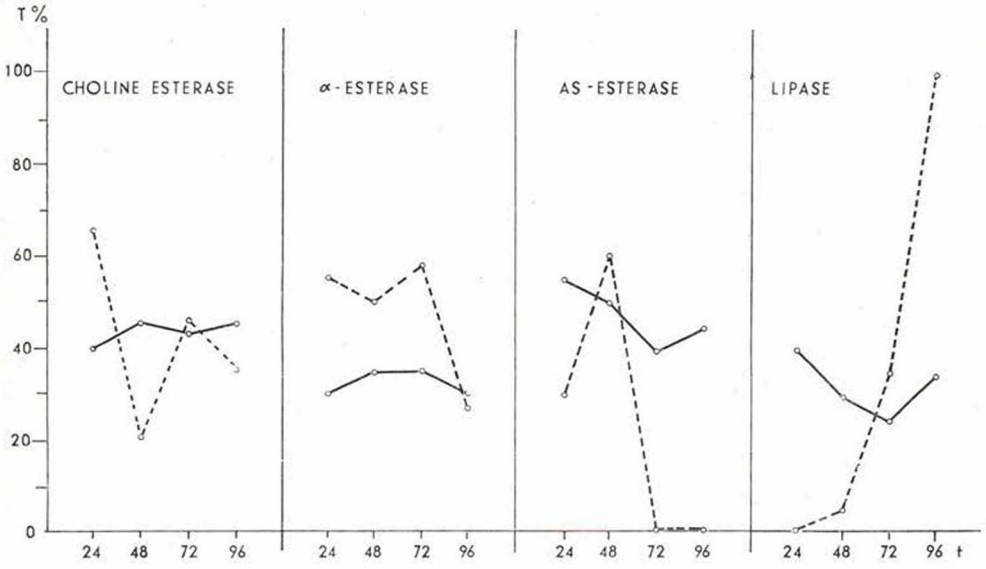

Fig. 1. Changes in esterase activity in cerebral ganglia as induced by treatment at 5 °C for 24, 48, 72, and 96 hours respectively.

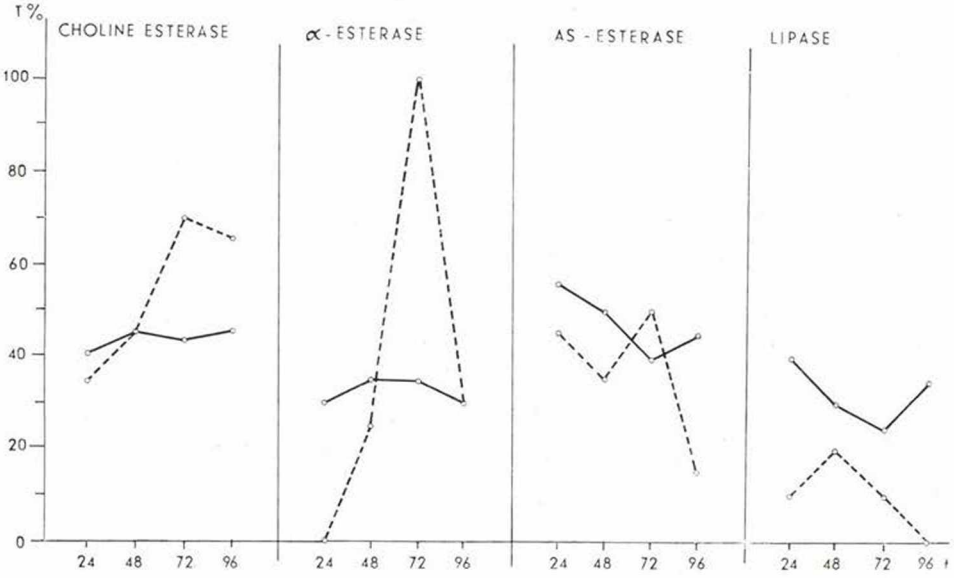

Fig. 2. Changes in esterase-activity in cerebral ganglia, as induced by treatment at 24 °C for 24, 48,72 and 96 hours, respectively.

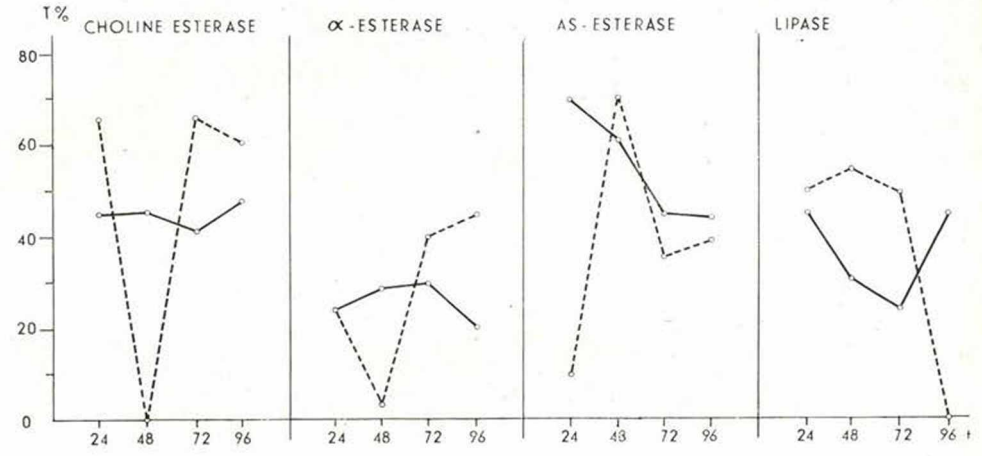

Fig. 3. Changes in estrase activity in visceral ganglia as induced by treatment at 5 °C for 24, 48, 72 and 96 hours, respectively.

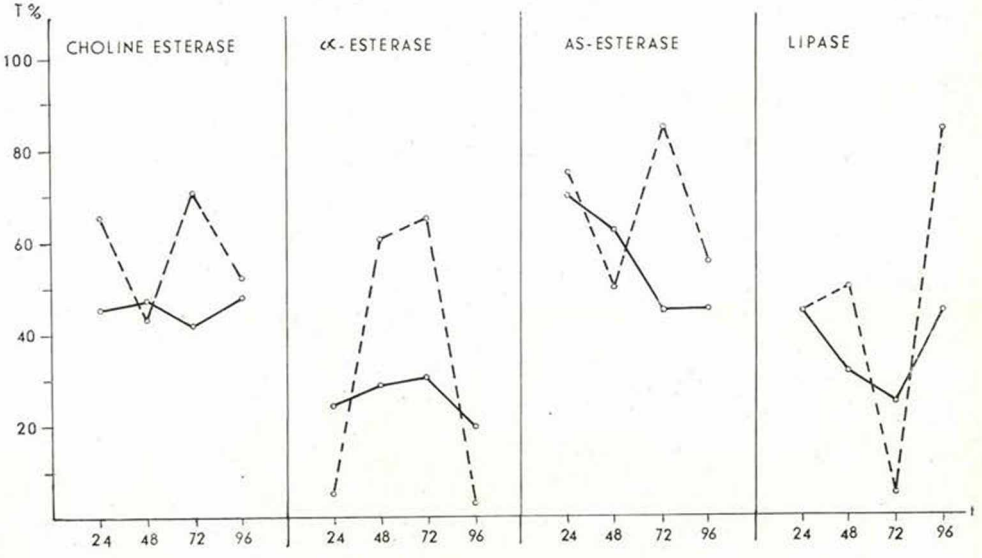

Fig. 4. Changes in esterase activity in visceral ganglia as induced by treatment at 24 °C for 24, 48, 72, 96 hours, respectively.

Alpha esterase The enzyme activity first decreased and then increased.

AS esterase. The enzyme activity decreased as a results of cold tretament.

Lipase. On the first 3 days lipase activity was around the control value, on the fourth day it decreased suddenly.

D) Changes in the visceral ganglia as induced by hot treatment (Fig. 4.)

Choline esterase. The enzyme activity varied in every 24 hours, but it remained generally above the control value.

Alpha esterase. The enzyme activity was sometimes significantly below and sometimes significantly above the control value.

AS esterase. The enzyme activity changed periodically every 24 hours

Lipase. The enzyme activity changed in every 24 hours, but the differences between the individual values were more significant than with AS esterase.

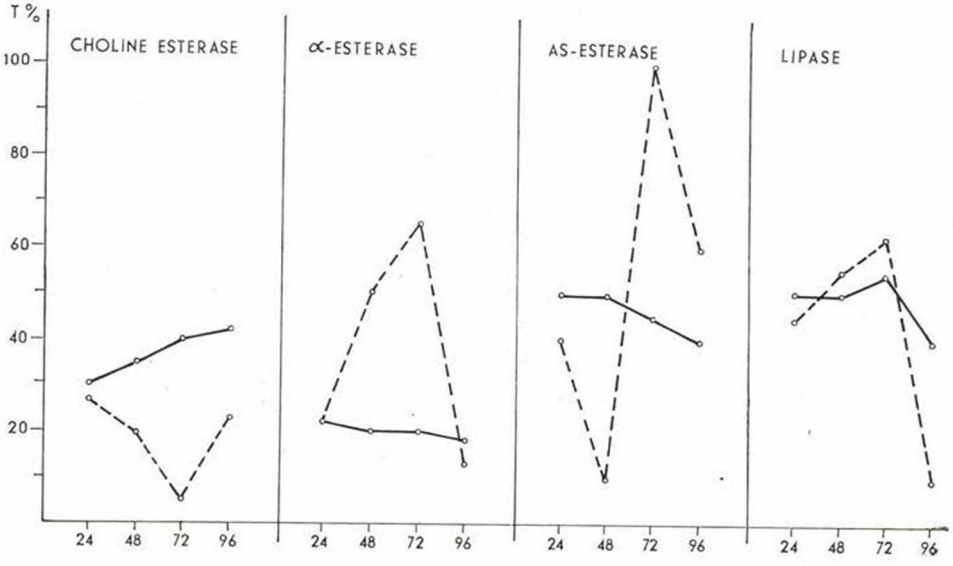

Fig. 5. Changes in esterase activity in pedal ganglia as induced by treatment at 5 °C for 24, 48, 72, 96 hours, respectively.

E) Changes in the pedal ganglia as induced by cold treatment (Fig. 5)

Choline esterase. The enzyme activity first decreased, and after 72 hours it increased again.

Alpha esterase. The enzyme activity increased as a function of time, but it decreased suddenly after 96 hours.

AS esterase. The enzyme activity varied every 24 hours. The deviations observed were very significant.

Lipase. The enzyme activities were first around the normal value, but after 72 hours they decreased significantly.

F) Changes in the pedal ganglia as induced by heat treatment (Fig.6)

Choline-esterase. The enzyme activity was well below the normal level, the nature of the curve changed completely.

Alpha esterase. On the first day the enzyme activity decreased significantly, then it increased, and it finally regained the normal level.

AS esterase. The enzyme activity decreased; the line of the curve varied significantly.

Lipase. The enzyme activity decreased significantly.

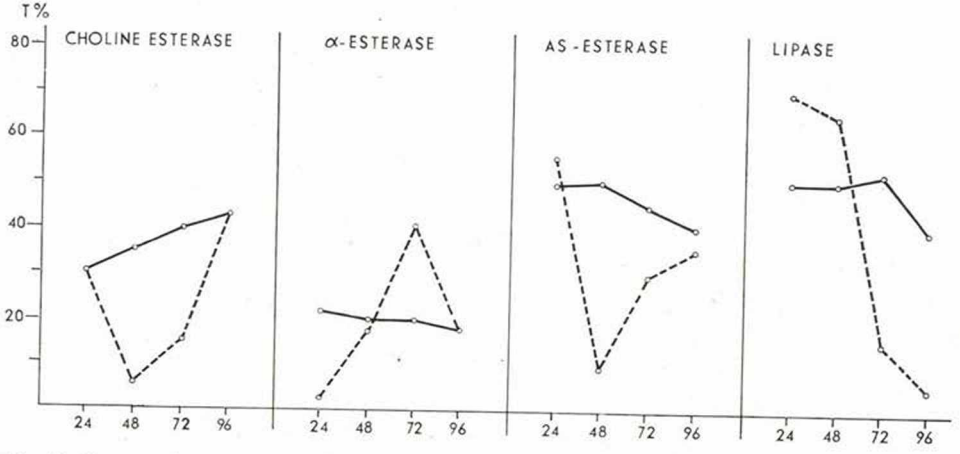

Fig. 6. Changes in esterase activity in pedal ganglia as induced by treatment at 24 °C for 24, 48, 72, 96 hours, respectively.

Discussion

The results show that the activity of the specific choline esterase and that of the non specific esterases such as alpha esterase and AS esterase change periodically in the control animals. It seems that the changes in esterase activity are connected with the rhythms in the life of the animals. Such a rhythm exists in the opening and closing of the valves, and in the neurosecretional cycles (Baranyi 1954, Baranyi – Salánky 1963), as well as during gametogenesis (Lubet 1955, Nagabushaman 1964). The neurosecretional cycle and the gametogenetic cycle show also an annual periodicity. But the periodicity of the opening and closing of the valves shows a 6 – 8-periodicity. As it seems on the basis of the experiments, this latter rhythmical is in connection with the changes in enzyme activity as affected by low and high temperatures.

Summary

The experimental results showed that the values of esterase activity in all three ganglia of the fresh-water mollusc were influenced by both cold (+5 °C) and heat (+24 °C) treatments. The activity of choline esterase proved to be the most sensitive to cold treatment; whereas that of lipase was most sensitive to heat treatment. It is probable that changes in the temperature acted as a sterss-effect on the ganglia which reacted to such effects by changes in the activity of several esterases.

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